REMARKS

Claim Amendments

By the present amendment, claims 1, 3 and 4 have been amended and claims 5-22 have been deleted. Claims 1-4 are pending in the application. The amendment does not contain new matter and has been made with prejudice. Please enter the amendment prior to calculating the claim fee.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Should the Examiner deem it beneficial to discuss the application in greater detail, she is kindly requested to contact Micheline Gravelle by telephone at 416-957-1682 at her convenience.

Respectfully submitted,

Gary Levy

Micheline Gravelle
Registration No. 40,261

Bereskin & Parr
Box 401, 40 King Street West
Toronto, Ontario, Canada
M5H 3Y2

(416) 364-7311

MG/jl
Encl.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In The Disclosure:

Paragraph beginning at page 1, line 1 has been amended to read as follows:

This application is a divisional of United States Patent Application Serial No. 09/442,143 filed November 15, 1999 (now pending), which is a continuation of PCT/CA98/00475, filed May 15, 1998, which claims priority to provisional application 60/061684 filed October 10, 1997 (now abandoned) and provisional applications 60/046,537 filed May 15, 1997 (now abandoned).

Paragraph beginning at page 4, line 35, has been amended to read as follows:

Figure 2 shows the nucleotide sequences of exon 1 of the mouse (SEQ.ID.NO.: 5) and human (SEQ.ID.NO.: 6) Fgl[2]3 genes;

Paragraph beginning at page 4, line 37, has been amended to read as follows:

Figure 3 shows the nucleotide sequences of exon 2 of the mouse (SEQ.ID.NO.: 7) and human (SEQ.ID.NO.: 8) Fgl2 genes;

Paragraph beginning at page 5, line 1, has been amended to read as follows:

Figure 4 shows the nucleotide sequence of the 3' UTR of hFgl2 (SEQ.ID.NO.: 9);

Paragraph beginning at page 5, line 2, has been amended to read as follows:

Figure 5 shows the amino acid sequences of the mouse (SEQ.ID.NO.: 4) and human (SEQ.ID.NO.: 2) Fgl2 proteins with the serine protease sites boxed;

Paragraph beginning at page 5, line 7, has been amended to read as follows:

Figure 8 shows the nucleotide sequence of the mouse (SEQ.ID.NO.: 10) and human (SEQ.ID.NO.: 11) Fgl2 gene promoter regions;

Paragraph beginning at page 5, line 9, has been amended to read as follows:

Figure 9 shows the nucleic acid sequence of the transcription binding sites in the putative promoter region of *hfgl2* (SEQ.ID.NO.: 12);

Paragraph beginning at page 5, line 22, has been amended to read as follows:

Figure 17 shows the Fgl-2 promoter DNA sequence (SEQ.ID.NO.: 13).

Paragraph beginning at page 7, line 7, has been amended to read as follows:

The present invention also provides an antibody that binds an epitope of hFgl2 comprising the amino acids at positions 300 to 400 in Figure 5. In a preferred embodiment, the present invention provides an antibody that binds an epitope of hFgl2 comprising the amino acids at positions 364-378 (DRYPSGNCGLYYSSG) (SEQ.ID.NO.: 18) in Figure 5.

Paragraph beginning at page 18, line 6, has been amended to read as follows:

As hereinbefore mentioned, the present inventor has cloned and sequenced genomic *hFgl2*. In this regard, the entire genomic sequence as well as the sequence of the promoter region, shown in Figure 8 (SEQ.ID.NO.: 11), and the 3' UTR, shown in Figure 4 (SEQ.ID.NO.: 9), are included within the scope of the invention.

Paragraph beginning at page 18, line 10, has been amended to read as follows:

Accordingly, in one embodiment the present invention provides an isolated nucleic acid molecule comprising (a) the sequence shown in Figure 8 (SEQ.ID.NO.: 11), where T can also be U; (b) nucleic acid sequences which have substantial sequence identity with (a); and (c) a fragment of (a) or (b).

Paragraph beginning at page 18, line 14, has been amended to read as follows:

In another embodiment the present invention provides an isolated nucleic acid molecule comprising (a) the sequence shown in Figure 4 (SEQ.ID.NO.: 9), where T can also be U; (b) nucleic acid sequences which have substantial sequence identity with (a); and (c) a fragment of (a) or (b).

Paragraph beginning at page 18, line 18, has been amended to read as follows:

The present invention also includes fragments of the nucleic acid sequences shown in Figure 2 or 3 or [SEQ.ID.NO.:1 or 3] SEQ.ID.NOS.:6 or 8 which have particular utility in the methods and compositions described above. The fragments generally comprise a nucleic acid sequence having at least 15 bases which will hybridize to the sequences shown in Figures 2 and 3 or [SEQ.ID.NO.:1 or 3] SEQ.ID.NOS.:6 or 8 under stringent hybridization conditions.

Paragraph beginning at page 19, line 7, has been amended to read as follows:

Further, it will be appreciated that the invention includes nucleic acid molecules comprising nucleic acid sequences having substantial sequence identity with the nucleic acid sequences shown in Figures 2 (SEQ.ID.NO.: 6), 4 (SEQ.ID.NO.: 9) and 8 (SEQ.ID.NO.: 11) and fragments thereof having at least 15 bases which will hybridize to these sequences under stringent hybridization conditions. The term "sequences having substantial sequence identity" means those nucleic acid sequences which have slight or inconsequential sequence variations from the sequences disclosed in Figures 2 (SEQ.ID.NO.: 6) and 3 (SEQ.ID.NO.: 8) , i.e. the sequences function in substantially the same manner to produce substantially the same activity as described herein for Fgl2. The variations may be attributable to local mutations or structural modifications. Nucleic acid sequences having substantial identity include nucleic acid sequences having at least 72%, preferably at least 75-95% identity with the nucleic acid sequences as shown in Figure 2 (SEQ.ID.NO.: 6) and Figure 3 (SEQ.ID.NO.: 8).

Paragraph beginning at page 19, line 18, has been amended to read as follows:

Isolated and purified nucleic acid molecules encoding a protein having the activity of human Fgl2 as described herein, and having a sequence which differs from the nucleic acid sequence shown in Figure 2 (SEQ.ID.NO.: 6) and Figure 3 (SEQ.ID.NO.: 8) due to degeneracy in the genetic code are also within the scope of the invention. Such nucleic acids encode functionally equivalent proteins (e.g., a protein having human Fgl2 prothrombinase activity) but differ in sequence from the sequence of Figure 2 (SEQ.ID.NO.: 6) and Figure 3 (SEQ.ID.NO.: 8) due to degeneracy in the genetic code.

Paragraph beginning at page 19, line 32, has been amended to read as follows:

The nucleic acid molecules of the invention can be used to isolate an *Fgl2* from other species. For example, a labelled nucleic acid probe based on all or part of the nucleic acid sequence shown in Figure 2 (SEQ.ID.NO.: 6) and 3 (SEQ.ID.NO.: 8) can be prepared, and used to screen an appropriate DNA library (e.g. a cDNA or genomic DNA library). Nucleic acids isolated by screening of a cDNA or genomic DNA library can be sequenced by standard techniques.

Paragraph beginning at page 20, line 19, has been amended to read as follows:

The initiation codon and untranslated sequences of human Fgl2 may be determined using currently available computer software designed for the purpose, (e.g. PC/Gene (IntelliGenetics Inc., Calif.). The nucleic acid sequence for a 3' untranslated region of hfgl2 is shown in Figure 4 (SEQ.ID.NO.: 9). The intron-exon structure and the transcription regulatory sequences of the gene encoding human Fgl2 may be identified by using a nucleic acid molecule of the invention encoding human Fgl2 to probe a genomic DNA clone library. Regulatory elements can be

identified using conventional techniques. The function of the elements can be confirmed by using them to express a reporter gene such as the bacterial gene lacZ which is operatively linked to the elements. These constructs may be introduced into cultured cells using standard procedures or into non-human transgenic animal models. Such constructs may also be used to identify nuclear proteins interacting with the elements, using techniques known in the art.

Paragraph beginning at page 20, line 31, has been amended to read as follows:

In addition to the full length amino acid sequence (Figure 5), the proteins of the present invention include truncations and analogs, and homologs of the protein and truncations thereof as described herein. A truncated Fgl2 protein or fragment of the human Fgl2 protein is a portion of the full-length Fgl2 amino acid sequence having one or more amino acid residues deleted. The deleted amino acid residue(s) may occur anywhere in the polypeptide, including at either the N-terminal or C-terminal end or internally. Fgl2 fragments typically will have a consecutive sequence of at least 10, 15, 20, 25, 30, or 40 amino acid residues that are identical to the sequences of the human Fgl2. The truncations or portions of the Fgl2 protein may comprise an antigenic site that is capable of cross-reacting with antibodies raised against the Fgl2 protein whose sequence is shown in Figure 5 (SEQ.ID.NOS: 2 and 4). Therefore, immunogenic portions or fragments of human Fgl2 proteins are within the scope of the invention (e.g. amino acids 300 to 400 in Figure 5). Preferably the truncated protein or portion of the protein binds with an affinity of at least about 10^6 L/mole to an antibody raised against human Fgl2.

Paragraph beginning at page 21, line 14, has been amended to read as follows:

The proteins of the invention may also include analogs of human Fgl2 as shown in Figure 5 (SEQ.ID.NOS: 2 and 4) and/or truncations thereof as described herein, containing one or more amino acid substitutions, insertions, and/or deletions. Amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions involve replacing one or more amino acids with amino acids of similar charge, size, and/or hydrophobicity characteristics. When only conserved substitutions are made the resulting analog should be functionally equivalent to human Fgl2 as described herein. Non-conserved substitutions involve replacing one or more amino acids with one or more amino acids which possess dissimilar charge, size, and/or hydrophobicity characteristics.

Paragraph beginning at page 21, line 23, has been amended to read as follows:

One or more amino acid insertions may be introduced into the amino acid sequence as shown in Figure 5 (SEQ.ID.NOS: 2 and 4). Amino acid insertions may consist of single amino acid residues or sequential amino acids ranging from 2 to 15

amino acids in length. For example, amino acid insertions may be used to destroy the prothrombinase activity of the protein.

Paragraph beginning at page 21, line 27, has been amended to read as follows:

Deletions may consist of the removal of one or more amino acids, or discrete portions (e.g.amino acids) from the human Fgl2 amino acid sequence as shown in Figure 5 (SEQ.ID.NOS: 2 and 4). The deleted amino acids may or may not be contiguous. The lower limit length of the resulting analog with a deletion mutation is about 10 amino acids, preferably 100 amino acids.

Paragraph beginning at page 21, line 32, has been amended to read as follows:

The proteins of the invention also include homologs of human Fgl2 as shown in Figure 5 (SEQ.ID.NOS: 2 and 4)and/or truncations thereof as described herein. Such homologs are proteins whose amino acid sequences are comprised of the amino acid sequences of human Fgl2 regions from other species that hybridize under stringent hybridization conditions (see discussion of stringent hybridization conditions herein) with a probe used to obtain human Fgl2 as shown in Figure 5 (SEQ.ID.NOS: 2 and 4). It is anticipated that a protein comprising an amino acid sequence which is at least 72% preferably 75 to 90% similar, with the amino acid sequence shown in Figure 5 (SEQ.ID.NOS: 2 and 4) will exhibit prothrombinase activity.

Paragraph beginning at page 23, line 36, has been amended to read as follows:

Human genomic DNA from the liver was amplified by Polymerase Chain Reaction using primers specific to the human cDNA sequence obtained from GenBank, that corresponds to exon 2 of mouse fgl2 gene; the sense primer CAA AAG AAG CAG TGA GAC CTA CA (SEQ.ID.NO.: 14) (hufpl7) is at position 692, and the antisense primer TTA TCT GGA GTG GTG AAA AAC TT (SEQ.ID.NO.: 15) (huflp8) is at position 1133 of the human cDNA. The PAC library, from Genome Systems Inc. (St. Louis, Missouri), was screened using the single amplicon, of about 300 nucleotides in length, that was produced from the above Polymerase Chain Reaction. Three clones, namely 6359, 6360, and 6361 were found positive for this screening. The plasmids containing these three clones were purified using the Qiagen maxiprep DNA purification protocol. The quality of the purified DNA and the presence of the inserts were verified by digesting the plasmid with Not 1 restriction enzyme (Canadian Life, Burlington, Canada), and subjecting the samples to Clamped Homogenous Electric Field (CHEF) gel electrophoresis , at 120 angle, 6 Volts, 1-20 seconds ramp interval, 0.5X TBE, and run time of 18 hours.

Paragraph beginning at page 24, line 13, has been amended to read as follows:

The clone 6360 was chosen for the rest of the study because in a dot blot analysis it consistently hybridized to sense primer GCA AAC AAT GAA ACA GAG GAA A (SEQ.ID.NO.: 16) (huflp1) at position 100 and anti-sense primer at position ATT GCC CTA TTA GAT AAC GAA TAC (SEQ.ID.NO.: 17) (huflp2) at position 1400. In order to reduce the DNA into fragments of 5 to 10 kb, which is a convenient size range to work with, the 6360 clone was digested under sub-optimal conditions with the restriction enzyme Sau 3A (Canadian Life, Burlington, Canada). The appropriate digest condition was found by incubating 5 µg of DNA with 1 µl of 2 µ/µl, 0.5 µ/µl, and 0.1 µ/µl of Sau 3A for one hour, at 37°C in a total reaction volume of 20 µl and observing the size range of the DNA fragments on a CHEF gel; the run conditions are 1 to 10 seconds ramp interval, 4.5 volts, 120 angle, 0.5X TBE, and a run time of 16 hours. The 6360 clone was large scale restriction digested by proportionately increasing the amount of DNA, reaction volume, and the amount of enzyme, that is, 10 µg, 40 µl, and 2 µl respectively. The final products of the restriction digest were subjected to CHEF gel electrophoresis at the above conditions. The DNA band corresponding to 6-9kb was excised and fragments were extracted using the Gene Clean DNA purification kit (Bio/Can Scientific, Mississauga, Ontario). The fragments were ligated into the alkaline phosphatase (Pharmacia, Uppsala, Sweden) treated BamH1 site of the Bluescript II vector (Stratagene) and transfected into DH10B competent cells by electroporation. This Bluescript II library was screened using the huflp1 and 2 primers. The primers were labeled at the 5 end with gamma P32 by using the enzyme Polynucleatide Kinase (Pharmacia, Uppsala, Sweden); these primers were used to screen the Bluescript library. The clone J14 hybridized to both these primers and was used for the subsequent work.

Paragraph beginning at page 27, line 24, has been amended to read as follows:

An AP1 site is located about 20 nucleotides from the TATA box (Figures 8 and 9). The consensus for AP1 motif is TGASTCA (SEQ.ID.NO.: 19), where S is a guanine or a cytosine. Except for the central S, cytosine in humans and guanine in mouse, the AP1 site is identical in mouse and human direct prothrombinase genes. AP1 is composed of dimers of proteins of the Fos and Jun proto-oncogene families. The Jun family members are DNA binding proteins; they bind to the AP1 site as homodimers or as heterodimers with Fos members. Upon activation, Jun gets dephosphorilated at a site proximal to DNA binding domain and acquires its ability to bind DNA (Curran and Franza, 1988; Woodgett et al., 1995). Furthermore, the transactivating domains of Fos and Jun get phosphorilated and are able to interact with the transcription machinery (Woodgett et al., 1995). In certain genes such as tissue factor gene, the AP-1 is required for both constitutive and induced expression (Mackman et al., 1989; Moll et al., 1995).

Paragraph beginning at page 33, line 25, has been amended to read as follows:

DNA from -3.5kb/+9bp and -1.3kb/+9bp fgl-2 promoter region pGL-2-Basic luciferase constructs (pL-3500, pL-1300) was obtained from clones previously constructed in Dr. Levy's lab (unpublished data). Additional 5' truncation series plasmids and the 3' pL3'274 luciferase vector were constructed first using PCR, followed by cloning into a PCR2.1 plasmid (Invitrogen). Specific portions of the pL-3500 clone were amplified at 35 cycles performed at 95 C for 1 min, 58 C for 1 min, 72 C for 2 min. The downstream 3' reverse primer, present in pGL2-Basic, was fixed for all 5' truncations and was 5'-GAA ATA CAA AAA CCG CAG AAG G-3' (SEQ.ID.NO.: 20) (Promega). The upstream primer used to construct pL-995 was 5'TCT TGG GAA ATC TGG TTA GAG-3 (SEQ.ID.NO.: 21). The upstream primer for pL-681 was 5'-GAG CTG AGT GAT GGG GAA GGA-3' (SEQ.ID.NO.: 22). The upstream primer for pL-294 was 5'-GGG CAC TGG TAT TAC AAC TGT-3' (SEQ.ID.NO.: 23), and the 5' primer for pL-119 was 5'-CTC CTC CTG TGT GGC GTC TGA-3' (SEQ.ID.NO.: 24). The fixed 5' forward primer for the 3' truncation was 5'-GGA TAA GGA GGG CAG GGT GAA-3' (SEQ.ID.NO.: 25). The downstream antisense primer for pL3'274 was 5'-ACA GTT GTA ATA CCA GTG CCC-3' (SEQ.ID.NO.: 26). Following PCR, PCR products were ligated and cloned into the PCR2.1 vector. PCR2.1 clones were sequenced to check for orientation, and DNA was obtained from desired clones. For the 5' truncations, the PCR2.1 clones were digested with KpnI and SalI, and then ligated and cloned into the pGL2-Basic luciferase vector (Promega) cut with KpnI and XhoI. Each final construct was checked with a specific diagnostic digestion before maxi-preps of DNA were made. For pL3'274, PCR2.1 clones were digested with EcoRV and HindIII, and then ligated and cloned into pGL2-Basic cut with SmaI and HindIII. A summary of the different constructs produced is shown in Figure 13.

Table 3 beginning at page 55 has been amended to read as follows:

PRIMER	SEQUENCE	5' POSITION	tm
			2(A+T)+4(G+C)
HUFLP1	GCA AAC AAT GAA ACA GAG GAA A (SEQ.ID.NO.: 16)	100	60
HUFLP2	ATT GCC CTA TTA GAT AAC GAA TAC (SEQ.ID.NO.: 17)	1399	64
HUFLP3	AAC GGA GAC CCA GGC AGA AAC (SEQ.ID.NO.: 27)	349	66
HUFLP4	CTT CGG GAG CTG AAT AGT CAA (SEQ.ID.NO.: 28)	243	62
HUFLP5	GAC AGC AAA GTG GCA AAT CTA (SEQ.ID.NO.: 29)	553	60
HUFLP6	TTC TGG TGA AGT TGG TGC TCC (SEQ.ID.NO.: 30)	832	64
HUFLP7	CAA AAG AAG CAG TGA GAC CTA CA (SEQ.ID.NO.: 31)	693	66
HUFLP8	TTA TCT GGA GTG GTG AAA AAC TT (SEQ.ID.NO.: 15)	1125	62
HUFLP9	TGA CCA AGA GTA AGG AAA TGA (SEQ.ID.NO.: 32)	908	58
HUFLP10	TGA CTG TAT TTG TTC TTG GCT G (SEQ.ID.NO.: 33)	639	62
HUFLP11	TTC TGG GAA CTG TGG GCT GTA (SEQ.ID.NO.: 34)	1134	64
HUFLP12	CCA GCT TCA TCT TTA CAG T (SEQ.ID.NO.: 35)	43	54
HUFLP13	AAT CAC TCT GTT CAT TCC TCC (SEQ.ID.NO.: 36)	1353	60
HUFLP14	GAA ATA ATA TGC ATT GAA A (SEQ.ID.NO.: 37)	-173	36
HUFLP14R	AAC GCA CAG GAA GAG GAG A (SEQ.ID.NO.: 38)	-96	58
HUFLP15	TTG ACA TCC TTT GAG ATA T (SEQ.ID.NO.: 39)	1459??	50
HUFLP16	ATG GGG CAT TGG GGA GC (SEQ.ID.NO.: 40)	-427	56
HUFLP17	GGC TAT CTC CTC TTC CTG T (SEQ.ID.NO.: 41)	-118	58
HUFLP18	TGA GCT ATG CCA GTG TCT GT (SEQ.ID.NO.: 42)	-755	60
HUFLP19	CAA GCG TAG TAT ACC AAA T (SEQ.ID.NO.: 43)	-288	52
HUFLP20	AAG GCA GGA AAG AGG AAC (SEQ.ID.NO.: 44)	-961	54
HUFLP21	GAC AAA GGA ATA GAA AGT AGC (SEQ.ID.NO.: 45)	-601	58
HUFLP22	CAG GGC AAA AAT CTA AAT G (SEQ.ID.NO.: 46)	-1092	52
HUFLP23	GCC CAG AGA GCA GGT AGA A (SEQ.ID.NO.: 47)	-883	60
HUFLP24	CCA GCC AGG GTT GAA ATA (SEQ.ID.NO.: 48)	3' end	54
HUFLP25	GCC CTG TCA GTC ATT TTG (SEQ.ID.NO.: 49)	promoter:not used	54
HUFLP26	AAA AAC CTA CCA GTA GTC T (SEQ.ID.NO.: 50)	3' end	52
HUFLP28	TTG GGG TGA CAT TAT GC (SEQ.ID.NO.: 51)	2399	50
HUFLP 29	TGA GCA GCA CTG TAA AGA TG (SEQ.ID.NO.: 52)	16	58
HUFLP30	GTG GCT TAA AGT GCT TGG GT (SEQ.ID.NO.: 53)	1350	60

<u>In The Claims:</u>

Claims 1, 3 and 4 have been amended as follows:

1. (Amended) A method of preventing or treating [a condition requiring a reduction in immune coagulation] <u>graft rejection</u> comprising administering an effective amount of an inhibitor of Fgl2 to an animal in need thereof.

3. (Amended) A method according to claim 2 wherein the antibody is a monoclonal antibody that binds to a human Fgl2 having the amino acid sequence as shown in [Figure 5] <u>SEQ.ID.NO.: 2</u>.

4. (Amended) A method according to claim 3 wherein the antibody binds an epitope of human Fgl2 comprising the amino acids [at positions 364-378 (]DRYPSGNCGLYYSSG[)] <u>shown</u> in [Figure 5] <u>SEQ.ID.NO.: 18</u>.

Claims 5-22 have been deleted.